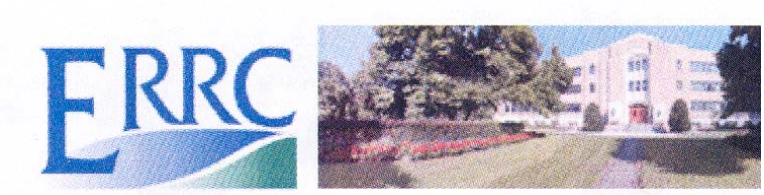

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Title: Radiation Sensitivity and Recoverability of *Listeria monocytogenes* and *Salmonella* on 4 Lettuce Types

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Citation: Journal of Food Science (2003) 68:(9) 2784-2787

Number: 7432

Radiation Sensitivity and Recoverability of *Listeria monocytogenes* and *Salmonella* on 4 Lettuce Types

B.A. NIEMIRA

ABSTRACT: *Listeria monocytogenes* or *Salmonella* was inoculated onto Boston, Iceberg, Green leaf, and Red leaf lettuces. Samples were γ-irradiated, and the radiation sensitivity of the inoculated bacteria determined. Recovery of bacteria from nonirradiated leaf pieces was also measured. Although the radiation sensitivity of *L. monocytogenes* was not influenced by the associated lettuce type, *Salmonella* was significantly less sensitive on Green leaf lettuce than on Boston, Iceberg, or Red leaf lettuces. For each pathogen, the recoverability from inoculated leaf pieces was significantly different among the 4 lettuce types; the pattern of recovery of *L. monocytogenes* was distinct from that of *Salmonella*. The antimicrobial efficacy of irradiation on inoculated lettuce was influenced by relatively subtle differences between lettuce types.

Keywords: irradiation, gamma, D value, radiation pasteurization, *Lactuca sativa*, variety

Introduction

Fresh produce has been associated with numerous outbreaks of foodborne illness in North America in recent years (Beuchat 1996). Salad vegetables, including fresh-cut lettuce, can be a source of pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella*, and *Shigella* spp. (Tauxe and others 1997; Gombas and others 2003; Horby and others 2003). *E. coli* O157:H7 is known to grow on shredded lettuce stored at 12 °C (Abdul-Raouf and others 1993). Ionizing radiation can effectively eliminate human pathogens from leafy salad vegetables such as lettuce (Foley and others 2002; Niemira and others 2002) and endive (Niemira and others 2003). The radiation sensitivity of bacteria can be influenced by the substrate upon which it is inoculated, such as different types of meats (Thayer and others 1995), various meat-based frankfurter formulations (Sommers and Thayer 2000), or different species of sprouts (Rajkowski and Thayer 2000). Published studies suggest that, even within a single commodity such as lettuce (Hagenmaier and Baker 1997; Prakash and others 2000; Niemira and others 2002), potato (Al-Kahtani and others 2000), or blueberry (Miller and others 1994; Miller and others 1995; Miller and McDonald 1996), the radiation sensitivity of associated bacteria or the product sensorial response may vary with commodity variety or subtype.

The objectives of this study were to determine the influence of lettuce type on (a) the radiation sensitivity of *Salmonella* and *L. monocytogenes* on lettuce leaves, (b) the attachment and recoverability of inoculated (nonirradiated) *Salmonella* and *L. monocytogenes* from leaf surfaces.

Materials and Methods

Pathogen

Pathogen cocktails were prepared from 2 outbreak isolates per genus. Archive cultures of *L. monocytogenes* isolates ATCC 49594 and ATCC 43256 (American Type Culture Collection, Manassas, Va., U.S.A.) and *Salmonella* strains *S.* Anatum F4317 and *S.* Stanley H0558 (Centers for Disease Control and Prevention, Atlanta, Ga., U.S.A.) were maintained on 50% glycerol at –70 °C. For each isolate, a culture from the frozen stock was regrown in tryptic soy broth (TSB, Difco, Detroit, Mich., U.S.A.) for 16 h at 37 °C with agitation and streaked onto tryptic soy agar (TSA, Difco). This was incubated at 37 °C for 24 h (*Salmonella*) or 48 h (*L. monocytogenes*) to form single colonies. These colonies were used to inoculate fresh TSB for each experiment and grown for 16 h at 37 °C with agitation. Aliquots of 100 mL of starting culture from each of the 2 isolates were mixed with 1800 mL of sterile BPB to make the working inoculum, with a final volume of 2000 mL. The cell density of the starting inoculum was determined by serial dilution with sterile Butterfield's Phosphate Buffer (BPB) (Applied Research Inst., Newtown, Conn., U.S.A.) and pour plating with TSA. Each isolate was sampled separately and after comingling. The cell density was typically 10^9 CFU/mL.

Lettuce

Fresh produce was obtained from local markets on the day of each experiment. Four types of lettuce were used: Boston (butterhead, somewhat compact head, deeply involuted leaves), Iceberg (crisphead, very compact head, relatively smooth leaves), Green leaf, and Red leaf (colored variants of looseleaf or bunching lettuce, oblong leaves). The outer leaves and any obviously damaged leaves of each head were removed and discarded, and cut leaf pieces were prepared. The basal portion of the head was removed, approximately 5 cm from the end. The leaves were sliced as a group into pieces weighing approximately 0.5 g.

Before use in the experiments, the cut leaf material was sanitized using a solution of 300 ppm sodium hypochlorite at room temperature. The leaf material was submerged and gently agitated for 3 min. The leaves were thoroughly rinsed under running distilled water and spun in a sterile salad spinner-type centrifuge (Oxo Intl., New York, N.Y., U.S.A.) to remove excess surface water. This design of salad spinner incorporates a container base, which captures all

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of the water removed from the leaf surface and prevents the formation of aerosolized droplets. The microflora of sanitized leaf material was measured for each lettuce type using a surface wash with BPB, serial dilution, pour plating with TSA, and incubation at 37 °C for 24 h. The post-sanitization population was found to be less than 20 colony-forming units (CFU)/g leaf tissue.

Inoculation

Leaf pieces were inoculated with either *L. monocytogenes* or *Salmonella* in separate experiments. The cut leaf pieces of each lettuce type were inoculated separately. Sanitized leaf pieces (approximately 500 g) were transferred to a sterile inoculation tub in a biological airflow hood, and the working inoculum was added. The material was agitated gently with a sterile slotted spoon for 120 s to completely submerge each piece, and then transferred to a sterile salad spinner-type centrifuge within the hood. The material was spun twice to remove excess inoculum from the surface of the leaf pieces. Samples (45 g) of each lettuce type were placed in nr 400 Stomacher bags (Tekmar, Inc., Cincinnati, Ohio, U.S.A.). The samples were refrigerated (4 °C) until irradiation, typically 30 to 60 min.

Irradiation

The inoculated leaf pieces were treated with 0.0 (control), 0.2, 0.4, 0.6, 0.8, or 1.0 kGy. In all cases, the irradiation was conducted at 4 °C. Temperature control was maintained during irradiation by the injection of gas coming from liquid nitrogen into the sample chamber. Each study was performed 3 times. The samples were irradiated using a Lockheed-Georgia (Marietta, Ga., U.S.A.) cesium-137 self-contained γ radiation source, with a dose rate of 5.64 kGy/h. The dose rate was established using alanine transfer dosimeters from the Natl. Institutes of Standards and Technology (Gaithersburg, Md., U.S.A.). Alanine pellets (Bruker, Inc., Billarica, Mass., U.S.A.) were used for dosimetry. The pellets were read on a Bruker EMS 104 EPR analyzer and compared with a previously determined standard curve. Actual dose was typically within 5% of the nominal dose.

Sampling

After irradiation, the samples were refrigerated until microbiological sampling, typically 60 to 90 min. The irradiated leaf material was examined by lab personnel for gross changes in color or texture. Sterile BPB (180 mL) was added to the stomacher bag and agitated for 60 s. The amount of BPB (180 mL) and the weight of inoculated leaf material (45 g) yields a final dilution factor of 1:5. A 1-mL sample was withdrawn for serial dilution with sterile BPB. The samples were diluted, pour plated with TSA (*Salmonella*) or Palcam (*L. monocytogenes*) (Difco). Three pour plates per dilution were incubated at 37 °C for 24 h (*Salmonella*) or 48 h (*L. monocytogenes*) and counted with an AccuCount 1000 automated counter (Biologics, Gainsville, Va., U.S.A.).

The data for each lettuce type were normalized against the control and plotted as the $\log_{10}$ reduction using the nominal radiation doses. The slopes of the individual survivor curves were calculated with linear regression using a computer graphics program (SigmaPlot 5.0, SPSS Inc., Chicago, Ill., U.S.A.). The ionizing radiation D_{10} value (the radiation dose necessary to inactivate 90% of the population) was calculated by taking the negative reciprocal of the survivor curve slope (QuattroPro, Corel Corp. Ottawa, Ont., Canada).

In a separate experiment, non-irradiated samples of cut leaf pieces, inoculated as described, were similarly sampled using sterile BPB. These samples were diluted and plated using TSA (*Salmonella*) or Palcam (*L. monocytogenes*). The plates were incubated at 37 °C for 24 h (*Salmonella*) or 48 h (*L. monocytogenes*) and counted; the data were taken to represent the recoverable bacterial counts, expressed as CFU/g leaf tissue. The study was performed 3 times. The data were also analyzed in terms of CFU/cm² leaf area, using previously published information on the surface area:weight ratio of these lettuce types: Boston, 25.6 mg/cm²; Green leaf, 29.2 mg/cm²; Iceberg, 35.9 mg/cm²; and Red leaf:19.9 mg/cm² (Niemira and others 2003).

Statistical analysis

The significance of differences between the slopes for the regression lines used to calculate the D_{10} values was determined with analysis of covariance (ANCOVA) (Excel, Microsoft Corp. Redmond, Wash., U.S.A.), using data pooled from the replications. The recoverability data were evaluated using analysis of variance (ANOVA, SigmaStat v. 4.0, SPSS, Inc.), using data pooled from the replications.

Results and Discussion

For both pathogens, significantly different numbers of CFU/g leaf tissue were recovered from the various lettuce types, but the range of variation was greater for *L. monocytogenes* than for *Salmonella* (Figure 1). Generally, the recovery from Boston and Green leaf was not different, whereas Iceberg had significantly fewer CFU/g than the other types. The extent to which Red leaf had significantly more CFU/g leaf tissue was pathogen-dependant. Some, but not all, of the significant variation was addressed by scaling the counts of recoverable bacteria against the surface area of the leaves (Figure 2).

Irradiation effectively reduced the population of *L. monocytogenes* (Figure 3) and *Salmonella* (Figure 4) on all 4 lettuce types. The radiation sensitivity of *L. monocytogenes* was not significantly influenced by the lettuce type. The D_{10} for *L. monocytogenes* was approximately 0.19 kGy (Table 1). In contrast, *Salmonella* was significantly less sensitive to irradiation when treated on green leaf

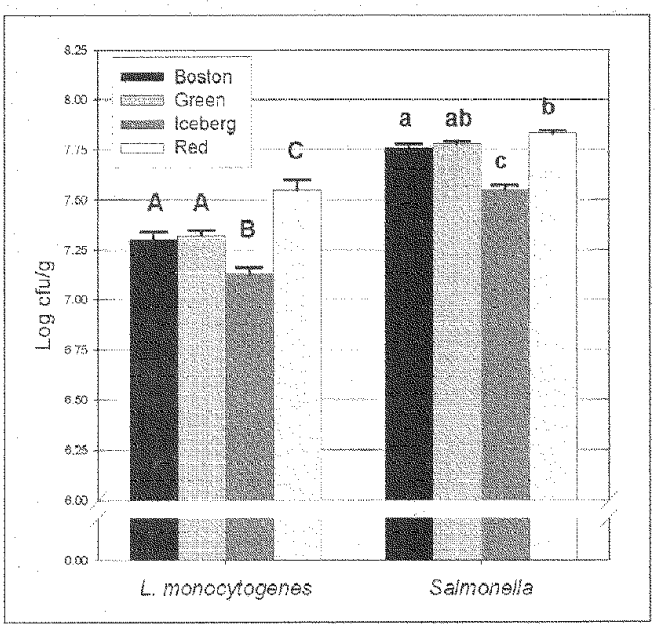

Figure 1—Recovery of *Listeria monocytogenes* and *Salmonella* from the surface of 4 lettuce types, in colony-forming units/g leaf tissue. Within each group, bars with different letters are significantly different ($P < 0.05$, analysis of variance). Error bars represent standard error ($n = 9$).

lettuce (D_{10} = 0.31kGy) than on any other type (D_{10} = 0.23 to 0.25 kGy) (Table 1).

On the basis of a visual and tactile inspection by lab personnel, there were no gross or readily apparent changes to the color or texture of the leaf pieces of any of the lettuce types, at any radiation dose used in the experiments.

Discussion

Ionizing radiation effectively reduced the level of *Salmonella* and *L. monocytogenes* on all of the lettuce types. The radiation sensitivity of inoculated *Salmonella* was significantly influenced by the type of lettuce with which it was associated, whereas *L. monocytogenes* was not. The influence of the suspending medium on radiation sensitivity of inoculated bacteria has been investigated using a variety of foods, including meats (Thayer and others 1995; Sommers and Thayer 2000) and produce (Rajkowski and Thayer 2000; Foley and others 2002; Niemira and others 2002; Niemira and others 2003); however, the underlying mechanisms by which the food substrate influences the associated bacteria are not well understood. A recent study, which used the same 4 types of lettuce used herein (Niemira and others 2002), found that the radiation sensitivity of an outbreak strain of *E. coli* O157:H7 was significantly influenced by the type of lettuce upon which it was inoculated. In that study, the D_{10} value obtained was significantly higher on the leaf pieces from compact head lettuces (Boston and Iceberg) than on the oblong head lettuces (Green leaf and Red leaf). That pattern was not observed in the current study, suggesting that the gross architecture of the lettuce head is not a good predictor of the relative radiation sensitivity of human pathogens. The specific determining factor or factors by which a given substrate influences bacterial radiation sensitivity may include the variations of moisture and gas composition seen on the smaller-scale architecture of the leaf surface, protective (or antagonistic) chemistries native to the product surface or originating from cut tissues, or some other, as yet unidentified factor.

Table 1—Radiation D_{10} values for *Listeria monocytogenes* and *Salmonella* on the surfaces of 4 types of lettuce

	Radiation D_{10} values (kGy ± SE)[a]	
Lettuce type	*L. monocytogenes*	*Salmonella*
Boston	0.19 ± 01a	0.24 ± 01a
Green leaf	0.19 ± 01a	0.31 ± 02b
Iceberg	0.20 ± 01a	0.25 ± 01a
Red leaf	0.19 ± 01a	0.23 ± 01a

[a]For each pathogen, values with different letters are significantly different ($P < 0.05$, analysis of covariance).

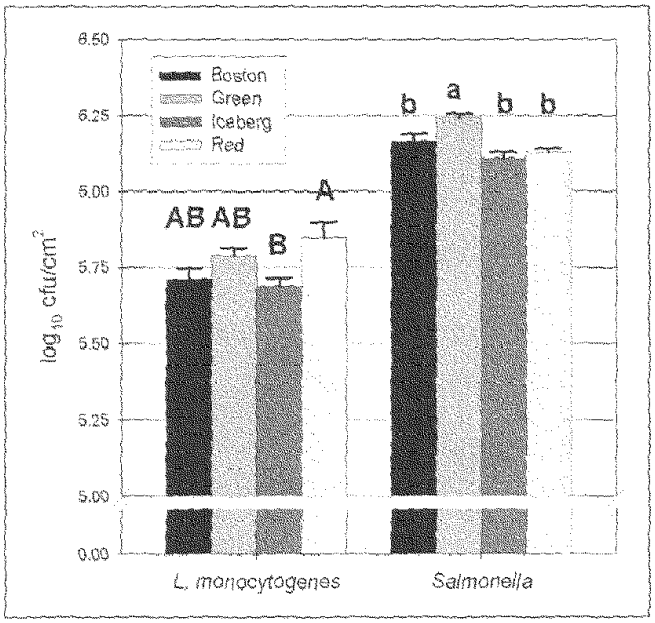

Figure 2—Recovery of *Listeria monocytogenes* and *Salmonella* from the surface of 4 lettuce types, in colony-forming units/cm² leaf tissue. Within each group, bars with different letters are significantly different ($P < 0.05$, analysis of variance). Error bars represent standard error (n = 9).

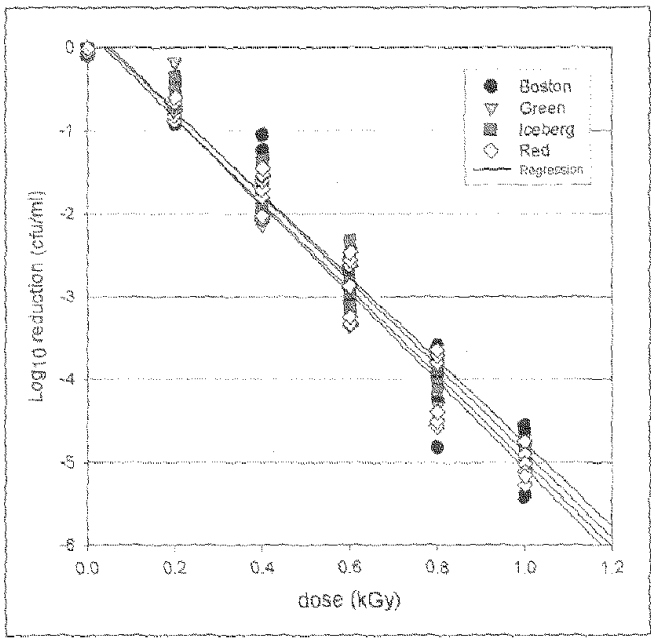

Figure 3—Radiation sensitivity of *Listeria monocytogenes* on the surfaces of 4 types of lettuce

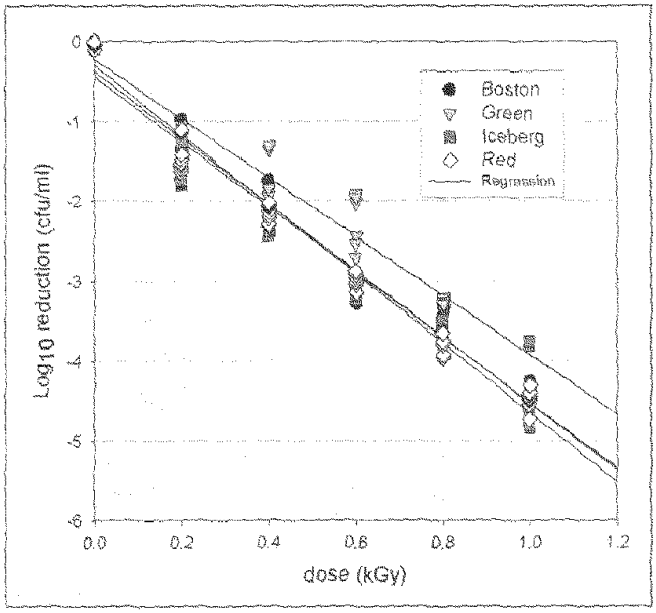

Figure 4—Radiation sensitivity of *Salmonella* on the surfaces of 4 types of lettuce

The radiation D_{10} values obtained for *L. monocytogenes* (approximately 0.19 kGy) and *Salmonella* (0.23 to 0.31 kGy) are generally consistent with published data for these pathogens (Rajkowski and Thayer 2000; Niemira and others 2003). The amount of radiation necessary to achieve a 5 $\log_{10}$ reduction is approximately 0.95 kGy with respect to *L. monocytogenes*, and 1.15 to 1.55 kGy with respect to *Salmonella*. Irradiation is known to cause softening in fruits and vegetables by radiolytic degradation of pectins (Yu and others 1996; Prakash and others 2002). As different commodities have different tolerances for irradiation, the dose below which no radiation-induced damage occurs is commodity-specific. The radiation doses used in this study did not cause any gross or readily apparent changes in color or texture of the leaf pieces for any of the 4 lettuce types. This result agrees with previously reported studies in which doses up to 0.5 kGy have little effect on the quality of lettuce (Hagenmaier and Baker 1997; Foley and others 2002; Niemira and others 2002; Fan and others 2003), and doses up to 0.8 kGy had little effect on endive, a leafy salad vegetable (Niemira and others 2003). When combined with a warm (47 °C) water dip, fresh-cut iceberg lettuce treated with doses of up to 1 kGy showed no significant loss of texture (Fan and others 2003). Chlorination plus irradiation (0.55 kGy) resulted in a 5.4 $\log_{10}$ reduction of *E. coli* O157:H7 on shredded iceberg lettuce with little significant effect on quality (Foley and others 2002). Although irradiation is clearly effective as a single antimicrobial treatment, it is generally accepted that irradiation should be part of an overall sanitation strategy that uses multiple interventions or "hurdles" to eliminate pathogenic bacteria while preserving product quality.

The 4 lettuce types examined differed significantly in the levels of bacteria recovered from inoculated leaf pieces. The pattern of recovery of *L. monocytogenes* based on CFU/g leaf tissue is the same pattern reported for *E. coli* O157:H7, that is, Iceberg < Boston = Green < Red (Niemira and others 2002). The pattern of recovery (CFU/g) of *Salmonella* is more variable. When scaled against the surface area of the leaves to yield CFU/cm^2, the patterns of recovery of neither *L. monocytogenes* nor *Salmonella* resemble the data published for *E. coli* O157:H7. The importance of such factors as differences in product topology has been raised by Beuchat and others (2001) in considering the various ways in which data may be presented, for example, CFU/g compared with CFU/cm^2. Factors such as hydrophobicity, stomatal density, trichome density, or other physical, chemical, or anatomical factors may influence bacterial association. Based on the varying response of the pathogens in the current study and in the recent work examining *E. coli* O157:H7 (Niemira and others 2002), it is clear that to this list of key factors must be added the specific type of pathogen of interest.

Conclusions

The results of this study support a growing body of literature that suggests that the relatively subtle differences among cultivars or types of the same commodity can have an important impact on the radiation sensitivity of associated pathogens, as well as on the physiological and sensorial response of the product to the irradiation process. Studies that examine the varietal response to irradiation of lettuce (Hagenmaier and Baker 1997; Niemira and others 2002), potato (Al-Kahtani and others 2000), and blueberry (Miller and others 1994; Miller and others 1995; Miller and McDonald 1996), along with the data presented herein, suggest that the sensory and microbiological response of fresh vegetables to irradiation processing is more strongly influenced by the type or cultivar than previously recognized. Therefore, the design of irradiation protocols for the sanitization of fresh-cut produce, such as leaf lettuce, should be validated for each separate product of interest.

Acknowledgments

Mention of brand or firm names does not constitute an endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned. The author would like to thank K. Lonczynski and K. Snipes for their technical assistance.

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